NOTES

Physicochemical Characterization of the Male-Specific RNA Bacteriophage $\mu 2$: Serological Comparison with R17, fr, and Q β

JEAN-CLAUDE PIFFARETTI* AND JEAN-SAMUEL PITTON

Institut Universitaire de Microbiologie Médicale, 22, Quai Ernest-Ansermet, 1211 Genève 4, Switzerland

Received for publication 5 March 1976

The physicochemical values and amino acid composition determined for bacteriophage $\mu 2$ suggest a close relationship with R17 and fr; significant differences are found with Q β ; these results are supported by serological studies.

For $\mu 2$, an RNA male-specific bacteriophage isolated by Dettori et al. (3), complete characterization is missing. The aim of this report is to fill this gap and to compare $\mu 2$ with R17, fr, and Q β .

Phages were grown in liquid medium, purified in SSC buffer (0.15 M NaCl plus 0.015 M sodium citrate, pH 7), and finally centrifuged in a CsCl density step gradient. Figure 1 shows the polypeptide pattern obtained with purified $\mu 2$ bacteriophage after electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide gels; as is usually found for RNA male-specific phages, $\mu 2$ contains a major and a minor protein, corresponding, respectively, to the coat and the maturation protein.

The number of copies of maturation protein per $\mu 2$ phage particle has been determined by SDS-polyacrylamide gel electrophoresis of ^{14}C -amino acid-labeled $\mu 2$ phages. Figure 2 shows the radioactivity pattern found; the label at the top of the gel probably comes from ^{14}C -labeled RNA. Krahn et al. (5) found, when labeling phages with radioactive amino acids, that part of the radioactivity incorporated is present in the RNA; on the other hand, the top of the gel could not be stained by Coomassie blue, suggesting the absence of protein (see Fig. 1). To determine the number of copies of maturation protein per phage particle, the following assumptions were made: (i) there are 180 copies of coat protein per phage particle (19); (ii) the molecular weights of the coat and maturation proteins are, respectively, 14,400 and 40,000 (see Table 1). A comparison of the theoretical Z values obtained from these data [$Z = [n \times 40,000]/[(n \times 40,000) + (180 \times 14,400)] = 1.52 \cdot 10^{-2}$ for $n = 1$ maturation protein copy, $3.03 \cdot 10^{-2}$ for $n = 2$, and $4.55 \cdot 10^{-2}$ for $n = 3$] with

the experimental Z' value estimated from Fig. 2 ($Z' = \text{radioactivity of the maturation protein peak} / \text{total protein radioactivity} = 1.53 \cdot 10^{-2}$) leads to the conclusion that maturation protein is present in one copy per $\mu 2$ phage particle.

Table 1 summarizes the physicochemical values obtained for phage $\mu 2$. The values found are similar to those reported for R17 and fr (11, 19) and for GA and SD (15): these RNA coliphages belong, respectively, to groups I and II, as defined by Watanabe et al. (14, 15); however, it should be noted that Q β and VK (15, 19), which belong to group III, have a higher sedimentation coefficient ($s = 84$) and a greater RNA molecular weight (molecular weight = 1.5×10^6 for Q β). From the pH_i value of the coat protein, it can be inferred that this protein is basic; this is confirmed by immunoelectrophoresis assays (data not shown) and by the amino acid composition (Table 2).

Table 2 shows the amino acid composition of $\mu 2$ coat protein; as for other RNA phages, it contains no histidine. The relatively high amount of valine, leucine, and isoleucine may help to explain the poor solubility of this protein in aqueous solutions (less than 1 mg/ml). Under our conditions, asparagine and glutamine are hydrolyzed as aspartic and glutamic acids; however, the proportions of these amino acids found in the amide form in R17 and fr (16, 18), associated with the amount of the basic amino acids arginine and lysine, may explain the high pH_i value determined for $\mu 2$ coat protein. The amino acid composition reported for R17 and fr (16, 18) is similar to the values obtained for $\mu 2$, whereas Q β , VK, GA, and SD (4, 8) present more significant differences (lack of histidine, tryptophan, and methionine for Q β and VK; lack of histidine, cysteine, and

FIG. 1. SDS-polyacrylamide (10%) gel electrophoresis of phage $\mu 2$. Gels were prepared by the method of Weber and Osborn (17). Phage samples were

FIG. 2. SDS-polyacrylamide (10%) gel electrophoresis of ^{14}C -amino acid-labeled phage $\mu 2$ (17). At the end of electrophoresis, gels were sliced. The fractions obtained were digested in NCS solubilizer (Amersham) and their radioactivity was determined. Peak I corresponds to maturation protein and peak II corresponds to coat protein. Radioactivity shown at the top of the gel (first fractions) is discussed in the text.

methionine for GA and SD; different content for other amino acids).

From the data presented in Table 2, the molecular weight of $\mu 2$ coat protein has been estimated at 14,400.

The relationships between $\mu 2$ and R17, fr, and $Q\beta$ have been studied by serological techniques. Examination of the precipitation lines obtained in the Ouchterlony immunodiffusion assay (Fig. 3) shows that $\mu 2$ and R17 are immunologically related; there is also a relationship between $\mu 2$ and fr, but the latter seems to have some uncommon antigenic determinants with the former. The same pattern is found between R17 and fr. In contrast, $Q\beta$ has no detectable immunological relationship with $\mu 2$, fr, or R17.

Neutralization assays to the RNA phages with the homologous and heterologous antisera (Table 3) confirm the results of the Ouchterlony immunodiffusion technique: $Q\beta$ -specific antiserum cannot inactivate the infectivity of the other phages; reciprocally, heterologous antisera are inactive to $Q\beta$. Examination of the K' values shows that $\mu 2$ and R17 have some differences in their antigenic determinants, which could not be detected by the Ouchterlony technique.

boiled for 2 min at 100°C ; at the end of electrophoresis, gels were stained in a Coomassie blue solution and destained in an electrical field. The major band corresponds to the coat protein and the minor one corresponds to the maturation protein.

TABLE 1. *Physicochemical properties of bacteriophage $\mu 2$*

Property	Value
Phage	
Mol wt $\times 10^{-6}$	3.8 ± 0.2^a
	4.3 ± 0.3^b
$s_{20,w}$	76 ^c
Density (g/cm ³)	1.42 ± 0.1^d
Specific absorbance (260 nm)	7.7 ± 0.5^e
pH _i	4.0 ± 0.1
Protein	
Coat protein mol wt $\times 10^{-3}$	14.4 ^f
Maturation protein mol wt $\times 10^{-3}$	40.5 ^g
Coat protein pH _i	8.8 ± 0.1
RNA ^h	
%	29
Mol wt $\times 10^{-6}$	1.1 ± 0.1
Base composition	
Adenine	23.8
Uracil	27.1
Guanine	25.4
Cytosine	23.7

^a Phage molecular weight determined from the percentage and the molecular weight of RNA.

^b Phage molecular weight determined from the $s_{20,w}$ value (9).

^c The $s_{20,w}$ value was determined on a Beckman model E analytical ultracentrifuge with Schlieren optics.

^d The density was determined by centrifugation to equilibrium in a CsCl gradient. Fractions were collected and analyzed for infectivity, and the density was determined with a Zeiss refractometer.

^e The specific absorbance determination was made by weighing dry phage residues from a suspension of known optical density.

^f Coat protein molecular weight was estimated from the amino acid composition (Table 2).

^g Maturation protein molecular weight was determined by SDS-polyacrylamide (10%) gel electrophoresis (17), with the following standards: ovalbumin, alcohol dehydrogenase, fumarase, and β -lactoglobulin.

^h The percentage of RNA was estimated from the measure of the RNA (phosphate, ribose) and protein (Lowry) content. The RNA molecular weight was estimated by SDS-polyacrylamide (2.6%) gel electrophoresis (1), using the following standards: RNA from poliovirus (the kind gift of M. F. Paccaud, Institute of Hygiene, Geneva) and *Escherichia coli* 16S and 23S ribosomal RNA. RNA bands were detected, using a UV Gilford gel scanner. RNA base composition was determined by Ceppellini et al. (2), and the isoelectric point (pH_i) was determined by isoelectric focusing.

Watanabe et al. (14, 15) classified RNA coliphages into group I (R17, fr), group II (GA, SD), and group III (Q β , VK), according to their serological and biochemical properties; no signifi-

TABLE 2. *Amino acid composition of the coat protein of $\mu 2^a$*

Amino acid	Amt
Lys	6
Arg	4
His	—
Asp	16
Asn	
Thr	
Ser	9
Glu	14
Gln	12
Pro	
Gly	
Ala	7
Val	11
Met	16
Ile	15
Leu	2
Tyr	8
Phe	8
Cys	2
Try	4
	2

^a Coat protein extracts (13) were purified by gel filtration on Sephadex G-75; samples were hydrolyzed in 6.15 N HCl at 107°C for 24, 48, 72, and 96 h, (6, 7, 12).

TABLE 3. *Neutralization assays of the RNA phages by homologous and heterologous rabbit antisera*

Anti-serum to phage	Homologous K value ^a	K' value ^b with phage:			
		R17	$\mu 2$	fr	Q β
R17	13,700	1.0	0.67	0.60	—
$\mu 2$	14,540	0.81	1.0	0.88	—
fr	11,730	0.73	0.82	1.0	—
Q β	4,100	—	—	—	1.0

^a Rate constant for phage inactivation (10).

^b Ratio of the heterologous *K* to the homologous *K* value was determined to make comparison possible.

cant, common, antigenic determinants could be detected between the different groups. Therefore, from the results presented in this report, it can be inferred that $\mu 2$ belongs to the same phage group as R17 and fr, i.e., to RNA coliphage group I.

These data support the hypothesis of Scott (10): most of the RNA phages may be serological mutants of one parent strain.

This work was supported by the Fonds National Suisse de la Recherche Scientifique (grant 3.668.71).

We wish to thank Yvette Froment and Verena Kocher-Hofer for excellent technical assistance, Michel Maire for the amino acid analysis, Cyrus Ahmad-Zadeh for helpful discussion, and Pierre-François Spahr for criticism.

FIG. 3. Ouchterlony immunodiffusion assays with $\mu 2$, fr, R17, and Q β bacteriophages. Rabbit antisera are diluted 1:8. Antigen concentration, 1 mg/ml.

LITERATURE CITED

1. Bishop, D. H. L., I. R. Claybrook, and S. Spiegelman. 1967. Electrophoretic separation of viral nucleic acids on polyacrylamide gels. *J. Mol. Biol.* 26:373-387.
2. Ceppellini, M., R. Dettori, and F. Poole. 1963. Sex specific bacteriophages of *E. coli* K12. III. Physico-chemical characterization of phage μ 2. *G. Microbiol.* 11:9-13.
3. Dettori, R., G. A. Maccacaro, and G. L. Piccinin. 1961. Sex specific bacteriophages of *E. coli* K12. *G. Microbiol.* 9:141-150.
4. Konigsberg, W., T. Maiter, J. Katze, and K. Weber. 1970. Amino-acid sequence of the Q β coat protein. *Nature (London)* 227:271-273.
5. Krahn, P. M., R. I. O'Callaghan, and W. Paranchych. 1972. Stages in phage R17 infection. VI. Injection of A protein and RNA into the host cell. *Virology* 47:628-637.
6. Liu, T.-Y., and Y. H. Chang. 1971. Hydrolysis of proteins with p-toluene sulfonic acid. *J. Biol. Chem.* 246:2842-2848.
7. Mueller, J. M., J. G. Pierce, H. Davoll, and V. du Vignand. 1951. The oxidation of oxytocin with performic acid. *J. Biol. Chem.* 191:309-313.
8. Nishihara, T., I. Haruna, I. Watanabe, Y. Noru, and Y. Okada. 1969. Comparison of coat proteins from three groups of RNA phages. *Virology* 37:153-155.
9. Pitout, M. J., J. D. Conradie, and A. J. van Rensburg. 1969. Relationship between the sedimentation coefficient and molecular weight of bacteriophages. *J. Gen. Virol.* 4:577-583.
10. Scott, D. W. 1965. Serological cross-reactions among the RNA-containing coliphages. *Virology* 26:85-88.
11. Stavis, R. L., and J. T. August. 1970. The biochemistry of RNA bacteriophage replication. *Annu. Rev. Biochem.* 39:527-560.
12. Stein, W. H., and S. Moore. 1954. The free amino-acids of human blood plasma. *J. Biol. Chem.* 211:915-927.
13. Sugiyama, T., R. R. Heber, and K. A. Hartman. 1967. Ribonucleoprotein complexes formed between bacteriophage MS2 RNA and MS2 protein "in vitro." *J. Mol. Biol.* 25:455-463.
14. Watanabe, I., T. Miyake, T. Sakurai, T. Shiba, and T. Ohno. 1967. Isolation and grouping of RNA phages. *Proc. Jpn. Acad.* 43:204-209.
15. Watanabe, I., T. Nishihara, H. Kaneko, T. Sakurai, and S. Osawa. 1967. Group characteristics of RNA phages. *Proc. Jpn. Acad.* 43:210-213.
16. Weber, K. 1967. Amino-acid sequence studies of the tryptic peptides of the coat protein of the bacteriophage R17. *Biochemistry* 6:3144-3154.
17. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecylsulphate-polyacrylamide electrophoresis. *J. Biol. Chem.* 244:4406-4412.
18. Wittmann-Liebold, B., and H. G. Wittmann. 1967. Coat proteins of strains of two RNA viruses. *Mol. Gen. Genet.* 100:264-274.
19. Zinder, N. D. (ed.). 1975. RNA phages. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.